

activity according to the method described by Brasier, A.R. et al. (1989) in Biotechniques Vol. 7, 1116-1122, is used.

- (2) A kit for screening a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production)

A kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) is characterized by use of the transformant described above. Examples of the kit for determining a compound or its salt that promotes or inhibits UCP-2 promoter activity of this invention are as follows.

① Screening reagents

1. Cell culture medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 10% fetal calf serum (Gibco Co.)

2. Cell differentiation medium

Dulbecco's modified Eagle's medium (Gibco Co.) supplemented with 5% rabbit serum (Gibco Co.)

3. Plasmid for measurement of UCP-2 promoter activity  
pGL3-basic (Promega Co.) plasmid DNA carrying UCP-2 promoter sequence of this invention and a structural gene (e.g. luciferase gene) inserted downstream of the

UCP-2 promoter

4. Host cell line

MG-63 cells (osteosarcoma cell line, obtained from ATCC)

5. Test compounds

Aqueous solutions are stored at 4°C or -20°C, and diluted to 1  $\mu$ M with cell differentiation medium at use. Test compounds that are slightly soluble in water are dissolved in dimethylformamide, DMSO, and methanol.

② Screening method

Host cells are seeded in 96-well microplates at a

density of  $1 \times 10^5$  cells/well, and cultured in an incubator at 37°C in 5% CO<sub>2</sub> overnight.

The cells are transfected with 1 µg/well of plasmid for UCP-2 promoter activity measurement.

- 5        One hour after transfection, 0.1 ml of test compound is added to each well, and the cells are cultured in an incubator at 37°C in 5% CO<sub>2</sub> for 48 hours.

After culture, 0.1 ml of PicaGene LT (Toyo Ink Co.) is added to each well, stirred for five minutes, 10 and then the luminescence is measured using a 96-plate measurement system (Amersham-Pharmacia Co.).

- (3) A compound or its salt that promotes or inhibits UCP-2 promoter activity (e.g. a compound that promotes or inhibits heat production) obtained using the 15 screening method described in (1) and the screening kit described in (2)

- If a compound that promotes or inhibits UCP-2 promoter activity is found using the screening method described in (1) or the screening kit described in (2), 20 the compound may be used as a prophylactic or therapeutic drug for obesity syndrome because the compound increases or promotes heat production, and thus, the compound may be used as a radical therapeutic drug for lifestyle diseases (diabetes, hypertension, 25 hyperlipidemia). Therefore, the compound may be used as an antiobestic drug, an antidiabetic drug, a depressor, and an antihyperlipemic drug.

- When the compound reduces or inhibits the promoter activity, the compound may be used as an antipyretic 30 drug because the compound decreases or inhibits heat production.

- A salt of the compound obtained using the screening method or the screening kit described above includes pharmaceutically acceptable salt. For example, 35 salts formed with inorganic bases, organic bases,

inorganic acids, organic acids, and basic and acidic amino acids are used.

Preferred salts formed with inorganic bases include alkaline metal salts such as sodium salts and potassium salts, alkaline earth metal salts such as calcium salts and magnesium salts, and aluminum salts and ammonium salts, etc.

Preferred salts formed with organic bases include salts formed with trimethylamine, triethylamine, pyridine, picoline, 2,6-lutidine, ethanolamine, diethanolamine, triethanolamine, cyclohexylamine, dicyclohexylamine, and N,N'-dibenzylethylenediamine, etc.

Preferred salts formed with inorganic acids include salts formed with hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid, etc.

Preferred salts formed with organic acids include salts formed with formic acid, acetic acid, propionic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and benzoic acid, etc.

Preferred salts formed with basic amino acids include salts formed with arginine, lysine, and ornithine, etc., and preferred salts formed with acidic amino acids include salts formed with aspartic acid and glutamic acid, etc.

When the said compound or its salt is used as prophylactic and/or therapeutic drugs for the diseases described above, the preparation can be obtained by the conventional methods.

For example, the said compound or its salt can be orally administered as sugar coated tablet, capsule, elixir, and microcapsule, etc., or non-orally as